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THE STERILIZATION OF SPACE VEHICLES TO PREVENT EXTRATERRESTRIAL BIOLOGICAL CONTAMINATION

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INTRODUCTION

Speculation on the existence of extraterrestrial life is sufficiently commonplace to suggest that the concept is subtly imbedded in our social culture. However, it is difficult to verify the origin of the extraterrestrial life concept because of a tendency to ascribe original authorship of many ideas to antiquity.[1]

The discovery of life on any of the planets would be one of the most exciting events in human history. Satisfying society's general

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[1]According to P. Duhem, Le Système du Monde (Vol. 1, pp. 17-18, 1913) the Pythagorean Philolaus (5th century B.C.) postulated the existence of an inhabited anti-earth which was always in opposition to the earth in relation to the sun.

curiosity would, however, be only one facet of the discovery. The event would also have tremendous scientific interest because, next to the synthesis of living matter in the laboratory, it would be the most important step that could be made toward understanding of the problem of the origin of life. Systems based on nucleic acid and proteins as bearers of life may or may not be unique. This is one of the fundamental questions that the discovery of extraterrestrial life might answer.

The biological importance of the planets is not limited to detecting and studying life on them. Even if no life is found to exist, the opportunity to sample organic compounds on the planets might give some valuable clues to the origin of life. Sterile worlds may provide the information necessary to the understanding of the organic chemical processes that preceded the development of life on the earth.

Present knowledge (as well as the lack of it) of the planets Mars and Venus is compatible with the possibility both of an indigenous life and of the support and rapid proliferation of terrestrial microorganisms. The introduction of terrestrial organisms and contaminants might so distort the biology of either planet as to constitute a scientific catastrophe. The processes are irreversible and they make the search for life on other planets most sensitive to irremediable harm. If the earth were sterile, it would require only months or years to universally populate it with the descendants of a single cell. A common bacterium, E. Coli, has a mass of 10^{-12} grams and a fission interval of 30 minutes. Ideally, it would take 66 hours for

the progeny to attain the mass of the earth. The progeny never reach this magnitude principally because the food supply is insufficient. Nevertheless, this extrapolation illustrates that the exponential growth rate of bacteria is truly explosive and, therefore, the timescale of planetary biological distortion need not be long. Indeed, it could be considerably less than the time interval of earth-planet oppositions. Space probes which have any likelihood of a landing, intentional or accidental, should be subject to careful sterilization.

DEFINITION OF BIOLOGICAL CONTAMINATION

It is convenient to separate biological contamination into two kinds, pollution and infection. Biological pollution is meant to be a deposit of a large enough number of micro-organisms to be scientifically significant, as such, without further growth. Infection is meant to describe the growth of one or more viable organisms. Likewise, pollution can be divided into two categories; viable pollution, which does not grow by nature of its environment, and non-viable pollution.

Pollution

Pollution is a type of contamination that applies to the Moon, Mars, and Venus. Pollution would be most likely if a mammal were splattered on any one of these three bodies. For example, the Moon's area is 4×10^{13} square meters, and the intestines of a mammal can contain 10^{12} micro-organisms per kilogram. If the mammal died in flight, the putrified tract could contain 10^{13} micro-organisms per kilogram (1). Present techniques are capable of detecting one

micro-organism per square centimeter. These techniques could be immediately extended to detect one micro-organism per square meter. Future improvements in technique may increase the detecting sensitivity by a few orders of magnitude; therefore, a single probe leaving a residue of from 10^9 to 10^{10} dead bacteria could provide a misleading background noise for future investigators.[2]

Infection

Infection appears least likely on the Moon because water is the lowest common denominator of all known terrestrial organisms, and all present evidence of solvents on the Moon's surface is highly controversial (2, 3). The hypothesis that beneath the lunar surface material one would find both water traces and relics of primitive organisms is not so unreasonable as to warrant the immediate dismissal of the matter of infection.

Mars is arid by terrestrial standards; its polar caps consist of thin hoar frost, and dense terrestrial type water clouds have never been observed. However, the polar caps retreat and the equatorial dark areas advance with the onset of the Martian spring. The pressure (85 mb, or less) and temperature (200-300°K) are so low it is frequently supposed that the presence of liquid water on the surface is very rare. This point has been refuted (4, 5). If salts are present on the Martian surface, an anti-freeze mechanism can occur.

[2]These comments would be unnecessary but for the fact that many people (not all laymen) presuppose that no significant discoveries will be made until a man is landed on the planets.

It is reasonable to suggest that the dark areas of the planet contain salts, perhaps in the form of deposits left behind by dried-up seas.

Sinton (6, 7) has found three small absorption dips, at 3.43, 3.57, and 3.67 microns, associated with the dark areas of Mars. This suggests the presence of organic matter on Mars, but the question of its origin is an open question.

These few facts indicate Mars may be a promising subject, both for basic biological research and infection.

Theories of Venus are so varied, and the facts so few, it is imperative to be very cautious, at least in the early stages of exploration.

SPACE-FLIGHT ENVIRONMENT

On cursory examination, probe sterilization may appear to be unnecessary because the space-flight environment is so hostile to terrestrial organisms. Several self-sterilizing mechanisms which immediately suggest themselves are:

1) Ultraviolet radiation from the sun
2) Space vacuum
3) High temperatures on the Moon's surface
4) Heat of impact, or impact explosion on the Moon
5) Heat of entry into a planetary atmosphere

We shall discuss these in order.

Parts of the probe will never be exposed to sunlight. Ultraviolet radiation will destroy organisms which are nakedly exposed, but its penetrating power is so low that organisms can survive if surrounded by only a small group of dead ones.

Laboratory vacuum is employed to help preserve micro-organisms. There is, as yet, no knowledge on space vacuum being bactericidal. Perhaps this question can be answered in the near future by means of a satellite experiment.

The Moon and the Planets most likely have cracks and fissures on their surfaces which would protect organisms from exposure to high temperatures and ultraviolet radiation, (5, p. 306).

A probe hard-landed on the Moon would have an impact velocity of approximately 3 kilometers per second. This is not sufficient kinetic energy to melt or vaporize the probe on impact, but it is sufficient to scatter parts of the payload all over the Moon's surface if the initial impact were on a hard surface, such as a mountain. The orbital velocity of a satellite in the Moon's gravitational field is roughly 2 kilometers a second. It is contended that the probe would bury itself in the Moon's surface. We do not believe that any of the supporting arguments presented thus far are sufficiently convincing to be dogmatic.

A probe that unintentionally enters an atmosphere has a high probability of coming in at a shallow angle, which is the ideal approach for a successful landing (8, 9). The probe may shed a few parts during the planet fall, but the bulk of it would strike the surface. A successful descent on Mars would be comparatively simple because the tenuous atmosphere of Mars extends so far out from its surface. Furthermore, meteors have been found whose interiors show no evidence of having been heated appreciably (10). It is evident that the rigors of a space journey are not a reliable means of preventing biological contamination.

INTERNATIONAL DISCUSSION

CETEX (Committee on Contamination by Extraterrestrial Exploration,) representing the International Scientific Unions, has published two reports (11, 12) in an attempt to set a tone for developing a code of conduct in space research. These reports imply that, particularly as regards biological exploration, a purely national program does not have much chance of being fruitful.

The CETEX reports recommend the sterilization of space probes, but they do not suggest a procedure for sterilizing probes nor do they suggest what tolerances would be acceptable. In this paper, we discuss both an operational approach to sterilization and the value judgements that will have to be faced by the operational agencies responsible for launching space vehicles.

OPERATIONAL TACTICS

Sterilizing space probes is an engineering nuisance, however, the same ordeal has confronted surgical crews for quite some time. In both instances, anticipation of the task is necessary.

At this time, it is possible to anticipate and recommend four phases of payload sterilization for all deep space missions. They are, in sequence:

1. Sterile assembly of components, particularly heat sensitive ones,
2. Built-in sterilization of parts, particularly where traces of water are admissable,

3. Terminal sterilization,

4. Maintaining sterilization.

A microbiological testing procedure must also be integrated into the sterilization operations.

Terminal Sterilization

Phase three, terminal sterilization, is the most important operation and we shall discuss it first.

All known micro-organisms perish when subjected to dry steam at 160°C for twenty minutes (13). There is a time-temperature effect. Micro-organisms can survive much higher temperatures over a shorter period of time; such as, the flash temperatures in explosions. However, approximately 20% of the components that go into payloads with which we are now familiar cannot endure 160°C. A more general disinfectant for this purpose is ethylene oxide gas (14).

Ethylene oxide (C_2H_4O) is the simplest of the ethers. It is a very small molecule and therefore dissolves in many substances, such as rubber, plastic, and oil. As a result of these properties, under slight pressure ethylene oxide is quite penetrating, working its way into the small interstices of most components. It is non-corrosive, and its human toxicity is low.

Ethylene oxide is a few thousand times more effective as a sporicide than other powerful disinfectants (15). Viruses are more sensitive to ethylene oxide than many other organisms, whereas, they are much more resistant to radiation.

Ethylene oxide is inflammable in air in concentrations as low as 3%. However, a mixture of 10% ethylene oxide and 90% carbon

dioxide (sometimes called carboxide) is not inflammable even when infinitely diluted with air. This mixture at 2 atmospheres pressure and 25°C would sterilize most parts of the probe in four hours. The sterilization could take place in a polyethylene tent and left there to retain its sterility for quite some time.

This part of the sterilization technique is well established. The U. S. Chemical Corps has sterilized many pieces of delicate laboratory apparatus without damage. They have also sterilized Air Force bombers and a commercial aircraft, in which a vial of live polio virus was accidentally broken.

Gaseous sterilization will not prove effective on certain impenetrable components. For these parts (paper capacitors for example) heat sterilization or radiation can usually be employed.

It is impractical to sterilize an entire payload with radiation. It is useful for certain small, sealed heat-sensitive components such as mylar capacitors.

The radiation dose required for some specified degree of sterilization is proportional to the natural logarithm of the number of bacteria. For 10^5 bacteria per gram of material, a dosage of 10^6 to 10^7 rem is required for good sterilization depending upon the organism. Actually 10^5 bacteria is a very high bacteria loading for most payload materials.

The Jet Propulsion Laboratory selected some sealed heat-sensitive components for radiation treatment by the General Electric Corporation. Two packages of identical parts were exposed to 10^6 and 10^7 rem from

a Co^{60} source of gamma rays. A majority of these components withstood 10^7 rem. The most important exceptions were transistors and mercury cell batteries.

We estimate that, between gas, heat, and radiation (terminal sterilization), 95% of the payload parts can be readily sterilized without fear of degrading their performance characteristics.

Sterile Assembly

The removal of dust and foreign particles from the space probe eliminates a major source of biological pollution and it is, at the same time, an engineering virtue. (Most atmospheric pollution is borne by dust particles, except perhaps in closed rooms crowded with human beings.)

The washing and scrubbing of parts of the payload with water and detergents (or other more acceptable solvents) can reduce the number of microbes on the probe by three orders of magnitude.

Other aspects of sterile assembly include using compounds that are made sterile. Parts such as screws and bolts can be dipped in any of a number of sterilizing solutions. If screws and fitting holes are made to fit exactly, then care must be taken to sterilize before joining. Such fittings will remain sterile. If the fittings are not perfectly joined, the ethylene oxide gas will penetrate and sterilize these interstices.

Built-In Sterilization

Wherever possible, substances which are inimical to the well being of micro-organisms should be employed. Certainly, substances of biological origin, such as casein glue or shellac, should be avoided.

Recently, germicides that contain organo-metallic compounds as active ingredients have been used to disinfect hospitals. These substances might prove valuable during the fabricating of sealed components with parts that get slightly contaminated with handling. This reduction of the contamination load during the initial stages, provides an opportunity to attempt terminal sterilization by radiation at a considerably reduced dosage, something of the order of 10^4 rem.

Built-in sterilization is not so much a specific technique as it is a philosophy of preparation for terminal sterilization.

Maintaining Sterilization

Once the space probe is sterilized, it will be necessary to mount it on the rocket boosters. The technical problem is then one of keeping microbes from coming into contact with the probe.

The probe is encased in a protective metal shroud during the launch phase of the space flight. The shroud can be employed to house a disinfectant atmosphere throughout the count down and flight through the atmosphere. The disinfectant can be either carboxide, employed in the terminal phase, or a faster acting but less penetrating gas, such as beta-propiolactone or ethylene imine.

Testing Procedure

In the past, several identical payloads were made for each mission. If this policy can be continued, it will not be difficult to produce convincing statistical arguments as to whether or not the payload meets the desired sterilization standard. Difficulties may arise, however, when the payloads become larger and more expensive.

In this respect, it would be most practical to turn terminal sterilization and the sterilization certification over to an organization outside the space-flight groups. It would still be the space agency's responsibility to integrate this independent statistical estimate of sterilization with the other probabilities involved. This brings us to the problem of determining acceptable contamination tolerances.

BIOLOGICAL CONTAMINATION TOLERANCES

Now we get to the heart of the matter as it is not practical to pursue codes of conduct and to employ testing techniques unless the community places a subjective value upon what the biologists want to protect. Discussions of the ethics of contamination are made confusing by people who persist in believing that sterility is an absolute, to which only a yes or no answer applies.

The answer to the question of probe sterility can be given only in terms of probabilities. When large numbers of micro-organisms are subjected to lethal treatment, the live count drops off exponentially with time, or approximately so. The process is mathematically similar to the radioactive decay of an unstable nucleus. The death of a micro-organism has no clear-cut definition.

A group of biologists in the United States, including some of the nation's most eminent microbiologists, biochemists, and biophysicists, who are also sensitive to the engineering areas in space research, have given this problem some intensive thought.

For Mars and Venus, the consensus is that the probability of landing one viable organism should be less than one in a million. This means that if the probability of successfully impacting a probe were judged a priori to be one in a hundred it would be necessary to sterilize the payload to a tolerance of one chance in ten thousand that it have a live organism. We are investigating what degrees of sterilization can be expected as the space program evolves.

As previously indicated, the status of the Moon as a biologically interesting target is considerably more doubtful than that of the planets; therefore, it is more difficult to get an intuitive grasp of what tolerances are acceptable. We tenatively suggest that one chance in ten (perhaps one hundred) of a viable organism remaining on the probe be an acceptable infection tolerance. We also suggest that pollution be kept less than 10^8 dead organisms per probe for Moon and planetary shots.

These tolerance levels are submitted here for general evaluation, with the understanding that, as more information on the celestial bodies becomes available, the levels should be revised.

RECOMMENDATIONS AND CONCLUSIONS

Planetary biology is one of the most exciting areas of space exploration. The unnecessary destruction of potential information in this research field by contamination would be an uncultural event. It is feasible to sterilize probes in such a manner that the loss of information to future investigators is minimized. This can be

accomplished utilizing ethylene oxide, heat and radiation, accompanied by the sterile assembly of special components, as sterilizing agents.

Pollution tolerances should be kept to 10^8 dead bacteria per missile. Infection tolerances should be kept to less than 10^{-6} per missile for the planets and 10^{-1} for the Moon.

A molecular inventory, preferably in the form of payload duplicates, should be kept for each space flight. More information on the chemical composition of space-probe materials should be acquired.

An agency specially qualified to handle sterilization should perform the terminal disinfection and ascertain the degree of sterilization.

ACKNOWLEDGMENTS

American scientists have been very patient while rocket technicians have picked their brains for information of value to space research. It has been precisely by this technique that we accumulated the facts contained in this paper. We hope that, by recognizing the gravity of the problem, we have partially compensated for our lack of originality.

Numerous people working with the National Academy of Sciences have assisted us in formulating our ideas and we thank them all. In particular we want to mention Joshua Lederberg for his characteristic insight, and Charles Phillips for making his work known to us.

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